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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12P 41/00, 17/10 — A1

(11) International Publication Number: WO 00/20623

(43) International Publication Date: 13 April 2000 (13.04.00)

(21) International Application Number: PCT/US99/21436

(22) International Filing Date: 1 October 1999 (01.10.99)

(30) Priority Data:
09/165,951 2 October 1998 (02.10.98) US

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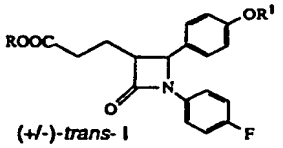
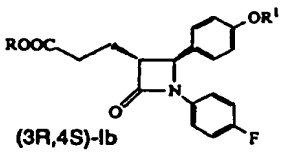
(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, HR, HU, ID, IL, IN, IS, JP, KG, KR, KZ, LC, LK, LR, LT, LU, LV, MD, MG, MK, MN, MX, NO, NZ, PL, PT, RO, RU, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

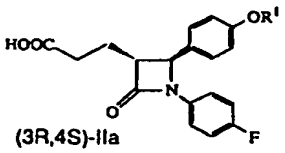
(54) Title: RESOLUTION OF TRANS-2 -(ALKOXYCARBONYLETHYL) -LACTAMS USEFUL IN THE SYNTHESIS OF 1-(4-FLUOROPHENYL) -3(R) -[3(S) -HYDROXY-3 -(4-FLUOROPHENYL) -PROPYL)] -4(S) -(4-HYDROXYPHENYL) -2-AZETIDINONE



(+/-)-*trans*- I (I)



(3R,4S)-Ib (Ib)



(3R,4S)-IIa (IIa)

(57) Abstract

A process for the microbiological or enzymatic hydrolytic resolution of racemic *trans*-2-(alkoxycarbonylethyl)-lactams of formula (I) wherein R is C_1-C_7 alkyl, 2,2,2-trifluoroethyl or methoxyethoxyethyl and RL is hydrogen or a protecting group is disclosed, whereby an optically enriched compound of formula (Ib) or (IIa) is obtained.

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RESOLUTION OF TRANS-2-(ALKOXYCARBONYLETHYL)-LACTAMS USEFUL IN THE SYNTHESIS OF 1-(4-FLUOROPHENYL)-3(R)-[3(S)-HYDROXY-3-(4-FLUOROPHENYL)-PROPYL)]-4(S)-(4-HYDROXYPHENYL)-2-AZETIDINONE

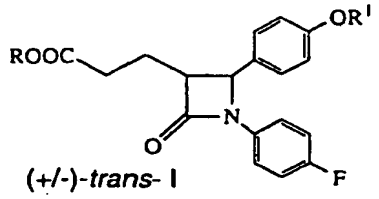
BACKGROUND OF THE INVENTION

Trans-2-(alkoxycarbonylethyl)lactams and *trans*-2-(carboxyethyl)lactams are intermediates in the synthesis of 1-(4-fluorophenyl)-3(R) -[3(S)-hydroxy-3-(4-fluorophenyl)-propyl)]-4(S)-(4-hydroxyphenyl)-2-azetidinone, a cholesterol lowering agent disclosed in US 5,767,115.

U.S. Patent 5,618,707 discloses stereoselective microbial reduction of a keto intermediate (4-(4-fluoro-benzoyl)butyric acid or a phenyloxazolidinone conjugate thereof) to the corresponding hydroxy intermediate used in the preparation of the azetidinone. Preferred microorganisms used in the process are *Zygosaccharomyces bailii* or *Schizosaccharomyces octosporus*.

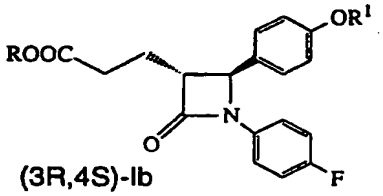
SUMMARY OF THE INVENTION

The process of the present invention relates to microbiological or enzymatic hydrolytic resolution of a racemic *trans* -2-(alkoxycarbonylethyl) lactam of the formula I:

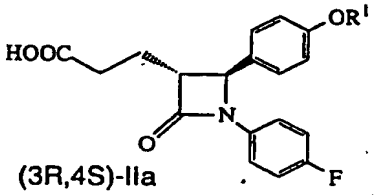


(+/-)-*trans*- I

wherein R is C_1-C_7 alkyl, 2,2,2-trifluoroethyl or methoxyethoxyethyl and R^1 is hydrogen or a protecting group selected from the group consisting of benzyl, trimethylsilyl, t-butyldimethylsilyl (TBDMS) and acetyl, to obtain an optically enriched compound of the formula Ib or IIa:



(3R,4S)-Ib



(3R,4S)-IIa

When a carboxylic acid ester of formula Ib is obtained, the process further comprises hydrolysis of the resulting compound of formula Ib to obtain an acid of formula IIa. The resulting 3R,4S lactam acid is useful as an intermediate in the preparation of 1-(4-fluorophenyl)-3(R) -[3(S)-hydroxy-3-(4-fluorophenyl)propyl)]-4(S)-(4-hydroxyphenyl)-2-azetidinone.

The resolution comprises the use of microorganisms (obtained from environmental sources and culture collections, e.g., the American Type Culture Collection (ATCC)) in medium, medium and buffer, medium and solvent, or medium and a mixture of buffer and solvent, or the use of enzymes in buffer, solvent or a mixture thereof, to which a racemic trans-2-(alkoxycarbonylethyl)lactam is added so that a compound having an ester or acid group of desired stereochemistry can be formed, accumulated and isolated. The resolution is either direct or subtractive, depending on the microorganism or enzyme used.

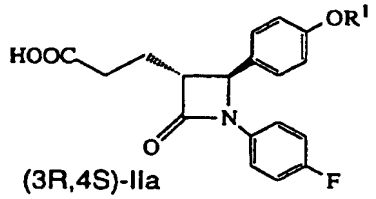
Microorganisms selected from the group consisting of the following genera have been found to be useful in the direct resolution: *Aspergillus, Bacillus, Candida, Cunninghamella, Debaryomyces, Mycobacterium, Paecilomyces, Penicillium, Rhodobacter, Streptomyces* and *Trichothecium*. The following species of the above genera are preferred: *Aspergillus alliaceus, niger, niveus* and *terreus*; *Bacillus sphaericus; Candida parapsilosis* and *rugosa*; *Cunninghamella homothallica; Debaryomyces hansenii; Mycobacterium fortuitum; Paecilomyces marquandii; Penicillium implicatum*; *Rhodobacter sphaeroides; Streptomyces spectabilis;* and *Trichothecium roseum.*

Microorganisms selected from the group consisting of the following genera have been found to be useful in the subtractive resolution: *Comamonas, Curvularia, Mucor, Nocardia* and *Rhodococcus.* The following species of the above genera are preferred: *Comamonas testosteroni; Curvularia brachyspora* and *geniculata; Mucor circinelloides* and *racemosus; Nocardia corallina;* and *Rhodococcus erythropolis, rhodochrous* and *species.*

Commercially available enzymes suitable for use in the resolution of this invention include Amano Lipase D (*Rhizopus delemar*); Amano Lipase FAP-15 (*Rhizopus javanicus*); Amano Lipase MAP-10 (*Mucor javanicus*); Amano Lipase N (*Rhizopus niveus*); Interspex Bacterial Esterase/Lipase BE1-Supported (*Pseudomonas mandocino*); Nagase Lipase A-10 (*Rhizopus japonicus*); Novo SP 525 (*Candida antarctica* , type B); Toyobo Lipoprotein lipase LPL-701 and LPL-311, type A (*Pseudomonas sp.*); Seikagaki Lipase (*Rhizopus delemar*); Kinzi & Payne Lipase WT (*Rhizopus sp.*); Svedas Lipase (*Rhizopus oryzae*); Sawa Lipase A-10 (*Rhizopus japonicus*); Sawa LPL-701 and LIP 301 (*Pseudomonas sp.*); Boehringer-Mannheim Chirazyme™ L2 (*Candida antarctica* lipase, type B); Boehringer-Mannheim Chirazyme™ L4 and L6 (*Pseudomonas sp.*); Interspex Lipase/Esterase ICS-16-FL1 Fungal (*Rhizopus oryzae*); Fluka Lipase (*Aspergilius niger*); Toyobo LIP-300/301 and LIP-321 (*Pseudomonas sp.*); Toyobo Lipoprotein lipase LPL 311 Type A (*Pseudomonas sp.*); Novo Lipozyme IM-60 (*Mucor miehei*); and Sigma Lipase Type XI (*Rhizopus arrhizus*).

Preferred enzymes are hydrolases of *Pseudomonas sp.* (Toyobo LPL 311 Type A, Toyobo LIP-301/LIP 300, Toyobo LPL 701, Boehringer-Mannheim Chirazyme™ L6).

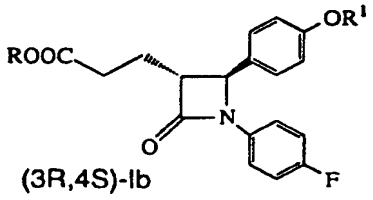
In particular, the present invention relates to direct resolution of trans-1-(4-fluorophenyl)-3-(alkoxycarbonylethyl)]-4(S)-(4-hydroxy-phenyl)-2-azetidinone comprising adding said compound to a micro-organism in medium, medium and buffer, medium and solvent, or medium and a mixture of buffer and solvent, especially wherein the microorganism is *Aspergillus terreus* or *alliaceus*, or *Candida parapsilosis*, incubating the resulting mixture, and isolating a compound of the formula IIa



(3R,4S)-IIa

wherein R^1 is as defined above.

In particular, the present invention also relates to subtractive resolution of trans-1-(4-fluorophenyl)-3-(alkoxycarbonylethyl)]-4(S)-(4-hydroxy-phenyl)-2-azetidinone comprising adding said compound to a microorganism in medium, medium and buffer, medium and solvent, or medium and a mixture of buffer and solvent, especially wherein the microorganism is *Rhodococcus rhodochrous* or *Rhodococcus species*, or to an enzyme in a solvent, buffer or a mixture thereof, especially wherein the enzyme is a hydrolase from *Pseudomonas sp.*, incubating the resulting mixture, and isolating a compound of the formula Ib:

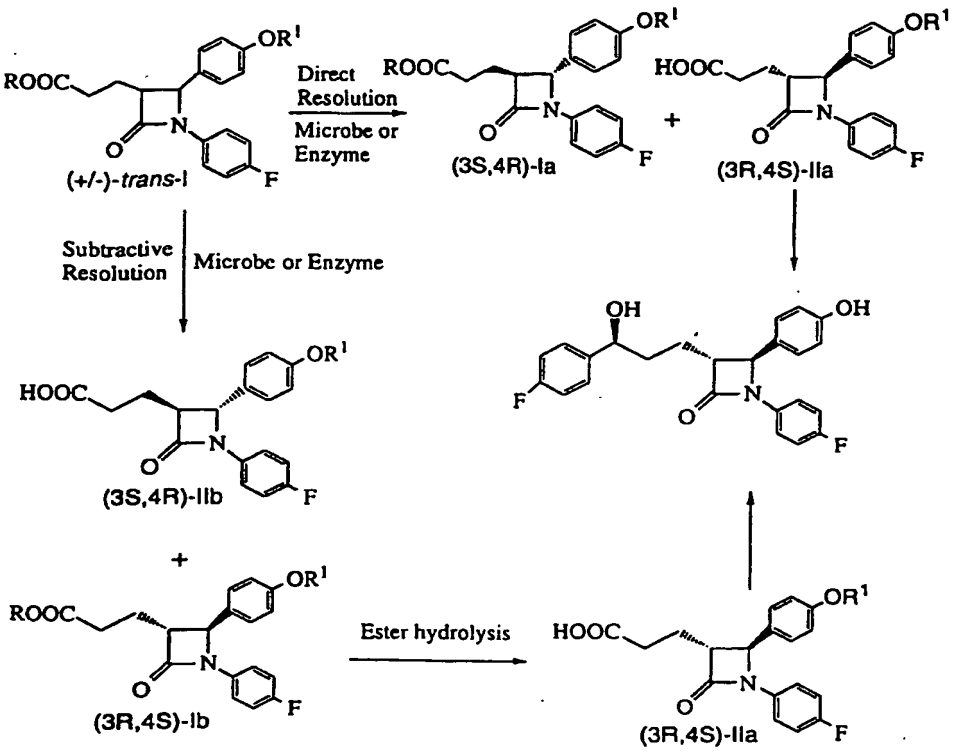


(3R,4S)-Ib

wherein R and R^1 are as defined above. The compound of formula Ib is then hydrolysed to remove the carboxylic acid ester group, R, to obtain a compound of formula IIa.

DETAILED DESCRIPTION

The hydrolytic resolution of the present invention is summarized in the following reaction scheme:



This scheme shows a method for performing a direct hydrolysis using a microorganism or enzyme, where racemic lactam ester I is hydrolyzed to generate enantiomerically enriched acid (3R,4S)-IIa which is easily separated from unreacted carboxylic acid ester (3S,4R)-Ia. Alternatively, a subtractive resolution of racemic lactam ester I yields acid IIb and enantiomerically enriched carboxylic acid ester (3R,4S)-Ib which is subsequently hydrolyzed to generate (3R,4S)-IIa. The enantiomerically enriched (3R,4S)-IIa is subsequently used to synthesize 1-(4-fluorophenyl)3(R)-[3(S)-hydroxy-3-(4-fluorophenyl)propyl)]-4(S)-(4-hydroxyphenyl)-2-azetidinone using procedures known in the art, for example by converting the acid of formula IIa to the corresponding acid chloride, reacting the acid chloride with a 4-fluorophenyl derivative, and reducing the ketone to the alcohol as described in Method H of 5,767,115.

The hydrolytic resolution is carried out by adding a racemic trans-2-(alkoxycarbonylethyl) lactam I to medium, medium and buffer, medium and solvent, or medium and a mixture of buffer and solvent containing microorganisms, or to solvent, buffer, or a mixture thereof, containing enzymes. The bioconversion may be conducted at temperatures in the range from between about 20°C to about 40°C; the microbial reaction is preferably conducted at ambient temperature to 30°C and the enzymatic reaction is preferably conducted at ambient temperature to 37°C. The initial pH value of the reaction is adjusted to be in the range from between about pH 5.0 to about 9.0, preferably pH 7.0.

The initial concentration of racemic *trans* lactam ester I in the microbial reaction may vary from between about 0.5 g/l to about 5 g/l, and is preferably 0.5 g/l. The duration of the microbial hydrolysis may vary from about 18 to about 96 hours, and is preferably about 48 hours.

The initial concentration of *trans* lactam ester I in the enzyme mediated reaction may vary from between about 5 mg/ml to about 200 mg/ml, and is preferably 25 mg/ml. The duration of the enzymatic hydrolysis may vary from about 24 to about 192 hours.

Suitable fermentation media, buffers and solvents are known to those skilled in the art. Fermentation media typically contain a carbon and nitrogen source or mixtures thereof, using such ingredients as yeast extract, nutrient broth, dextrose (cerelose), white potato dextrin, soy flour, peptone and other components known in the art. Typical buffers are phosphate buffer (e.g., 0.1 M at pH 7), MES (2-[N-morpholino]ethane-sulfonic acid), Bis-Tris (bis[2-hydroxyethyl]iminotris[hydroxymethyl]-methane), PIPES (1,4-piperazine-diethanesulfonic acid), HEPES (N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]), TRIS (tris(hydroxy-methyl)aminomethane) and MOPS (3-[N-morpholino]propanesulfonic acid) buffer (e.g., 0.1 M at pH 7). Typical solvents are acetonitrile, acetone, ethyl ether, isopropanol, t-butanol, isoamyl alcohol, p-dioxane, isopropyl ether, dimethyl sulfoxide, t-butyl methyl ether (TBME), toluene, tetrahydrofuran and CH_2Cl_2. Preferably, the microbial resolutions are carried out in fermentation media, and the enzymatic resolutions preferably are carried out in a buffer with a co-solvent; a preferred co-solvent for enzymatic resolutions is TBME.

At the end of the hydrolysis, optically enriched acids or esters may be extracted using organic solvents such as ethyl acetate (EtOAc), TBME, CH_2Cl_2 and the like. Adsorption to resins, chromatography, and other physical methods known in the art may also be used for the isolation of optically enriched acids or esters.

The carboxylic acid ester of formula Ib can be hydrolysed to the corresponding acid of formula IIa using methods well known in the art, for example by treatment with a suitable base, e.g., LiOH, as described in US 5,767,115.

The examples below demonstrate the evaluation of microorganisms and enzymes in the hydrolysis of this invention and the preparation of milligram quantities of compounds of formulas IIa and Ib.

Example 1

The general method for identifying the microbial hydrolysis of racemic *trans* lactam methyl ester I for use in generating acid IIa is described below.

Seed cultures of yeast, filamentous fungi, and bacteria were grown in 125 ml or 300 ml flasks containing 25 ml or 50 ml of YPD (1% yeast extract, 2% peptone, 2% dextrose; pH 5.5), SIM6 (3.5% soy flour, 5% white potato dextrin, 0.5% cerelose, 2mg/l cobalt chloride, 0.5% calcium carbonate; pH 6.0) and NYC (0.8% nutrient broth, 2% yeast extract, 2% cerelose; pH 7.0) media respectively, for 72 hours at 30°C with agitation (175-250 rpm) prior to inoculation (4 % v/v) into flask fermentations (25ml YPD/125 ml flask for yeast and filamentous fungi or 25ml NYC /125 ml flask for bacteria) which were incubated at 30°C with agitation (250 rpm). In all fermentations, medium pH was adjusted prior to inoculation but was not controlled during culture propagation and substrate hydrolysis. Microbial resolution was initiated by adding 0.5 g/l of racemic *trans* lactam methyl ester I dissolved in ethanol (25 mg/ml), directly to cultures following 24 hours of growth. Samples of fermentation broth were extracted with TBME following 24-72 hours incubation with substrate and were analyzed by reverse-phase HPLC. Preferred cultures demonstrating selective hydrolysis generating acid IIa are summarized in Table 1.

Table 1: Direct resolution of racemic *trans* lactam methyl esters using microorganisms

Culture	Strain # (ATCC)	Substrate	Product	% ee	% Yield
A. terreus	10020 20542 24839	benzyl protected racemate I	benzyl protected IIa: (3R,4S) acid	100 91 91	9 23 35
Penicillium implicatum	SPR 938*	benzyl protected racemate I	benzyl protected IIa: (3R,4S) acid	100	6
Aspergillus niger	9029	unprotected racemate I	IIa: (3R,4S) acid	100	14
Aspergillus alliaceus	1024	unprotected racemate I	IIa: (3R,4S) acid	100	29
Candida parapsilosis	7330 16632 22019 34078	unprotected racemate I	IIa: (3R,4S) acid	100 100 100 100	29 26 28 20
Candida rugosa	14830	unprotected racemate I	IIa: (3R,4S) acid	100	20

* Schering-Plough Research: Biotransformations Culture Collection

Example 2

Milligram quantities of acid IIa derived from the microbial hydrolysis of benzyl protected racemic *trans* lactam methyl ester I was prepared as described below.

Microbial resolution of methyl ester I (0.5 g/l) to generate acid IIa was conducted as described in Example1 using multiple flask fermentations employing *Aspergillus terreus* strain ATCC # 24839. Following 48 hours of incubation, fermentation broths of each of the cultures were pooled prior to centrifugation to separate the cells from the fermentation broth. Cell pellets were pulverized in liquid nitrogen using a mortar and pestle prior to three sequential extractions with TBME (1-2 volumes/wet weight). Fermentation broth was extracted separately with TBME. The TBME extracts contained both the (3R,4S)-acid and the (3S,4R)-ester, each in >99% enantiomeric excess. Anhydrous $MgSO_4$ was added to the TBME extracts to remove residual water, the extracts were filtered and the filtrate concentrated by evaporation. Extract concentrate was subjected to purification by preparative thin layer

chromatography employing multiple10-20 GF silica plates (20cmX20cmX1000 micron) and developed with a solution of EtOAc: hexane (50:50). Material comigrating with the desired product was scraped from each of the silica plates, pooled and eluted from the silica with TBME. The eluate was evaporated to yield the (3R,4S)-acid IIa: 170 mg, 17% yield; 86% enantiomeric excess; $[\alpha]_D^{25} = -13.0°$ (c = 0.123, ethanol).

Example 3

The general method for identifying the microbial resolution of benzyl protected racemic *trans* lactam methyl ester I for use in generating ester Ib is described below.

Seed cultures of yeast, filamentous fungi, and bacteria were grown in 125 ml or 300 ml flasks containing 25 ml or 50 ml of YPD (1% yeast extract, 2% peptone, 2% dextrin; pH 5.5), SIM6 (3.5% soy flour, 5% white potato dextrose, 0.5% cerelose, 2mg/l cobalt chloride, 0.5% calcium carbonate; pH 6.0) and NYC (0.8% nutrient broth, 2% yeast extract, 2% cerelose; pH 7.0) media respectively, for 72 hours at 30°C with agitation (175-250 rpm) prior to inoculation (4 % v/v) into flask fermentations (25ml YPD/125 ml flask for yeast and filamentous fungi or 25ml NYC /125 ml flask for bacteria) which were incubated at 30°C with agitation (250 rpm). In all fermentations, medium pH was adjusted prior to inoculation but was not controlled during culture propagation and substrate hydrolysis. Microbial resolution was initiated by adding 0.5 g/l of racemic *trans* lactam methyl ester I dissolved in ethanol (25 mg/ml), directly to cultures following 24 hours of growth. Samples of fermentation broth extracted with TBME following 24-72 hours incubation with substrate were analyzed by reverse-phase HPLC. Cultures yielding optically enriched ester Ib are summarized in Table 2.

Table 2: Subtractive resolution of racemic *trans* lactam methyl esters using microorganisms

Culture	Strain # (ATCC)	Substrate	Product (methylester)	% ee	% Yield
R. erythropolis	4277 11048 19369	benzyl protected racemate I	benzyl protected Ib: (3R,4S)	100 100 100	17 17 11
R. rhodochrous	29670 19150 29675	benzyl protected racemate I	benzyl protected Ib: (3R,4S)	100 100 100	30 22 24
R. species	19148 19071	benzyl protected racemate I	benzyl protected Ib: (3R,4S)	100 100	31 31
C. testosteroni	33083	benzyl protected racemate I	benzyl protected Ib: (3R,4S)	100	12
N. corallina	31338	benzyl protected racemate I	benzyl protected Ib: (3R,4S)	100	11

Example 4

Milligram quantities of methyl ester Ib derived from the hydrolysis of benzyl protected racemic *trans* lactam methyl ester I (0.5 g/l) was prepared as described in Example 3 using multiple flask fermentations employing *Rhodococcus species* ATCC # 19071. Following 48 hours of incubation, fermentation broths of each of the flasks were pooled prior to centrifugation to separate the cells from the fermentation broth. Cell pellets were disrupted by sonication prior to three sequential extractions with TBME (1-2 volumes/wet weight). Fermentation broth was extracted separately with TBME. Anhydrous $MgSO_4$ was added to the TBME extracts to remove residual water, the extracts were filtered and the filtrate concentrated by evaporation. Extract concentrate was subjected to purification by preparative thin layer chromatography employing multiple10-20 GF silica plates (20cmX20cmX1000 micron) and developed with a solution of EtOAc: hexane (50:50). Material comigrating with the desired product was scraped from each of the silica plates, pooled and eluted from the silica with TBME. The eluate was evaporated to yield the (3R,4S)-ester; 360 mg, 36% yield; >99% enantiomeric excess; $[\alpha]_D^{25} = -7.5^\circ$ (c = 0.133, ethanol).

Example 5

The general method for identifying the enzymatic resolution of benzyl protected racemic *trans* lactam methyl or trifluoroethyl esters I for use in generating optically enriched acid and ester is described below.

Enzyme screening reactions were conducted using a two-phase system of 0.6 ml TBME with 1.0 ml of 0.1 M phosphate buffer (pH 7.0). Enzyme, typically 50-200 mg or 100-200 µL, was added to the suspension followed by 14.4 mg of methyl ester. The mixture was agitated (350 rpm) at room temperature. Some deviations from these reaction conditions were evaluated as indicated in Table 3. Material was recovered by separating the phases by centrifugation and product and unreacted starting material were analyzed by chiral HPLC. Enzymes demonstrating selective hydrolysis of racemic *trans* lactam methyl ester I yielding acid IIb and ester Ib are summarized in Table 3.

Table 3. Enzymatic resolution of benzyl protected racemic *trans* lactam methyl ester I yielding optically enriched ester Ib and acid IIb.

Enzyme	Time (hour)	(3S,4R) IIb ee_p	(3R,4S) Ib ee_s	Conversion	E
Amano Lipase D *Rhizopus delemar*	64.5	0.71	0.67	0.485	11
Amano Lipase FAP-15 *Rhizopus javanicus*	44.25	0.76	0.49	0.392	12
Amano Lipase MAP-10 *Mucor javanicus*	64.5	0.68	0.39	0.364	8
Amano Lipase N *Rhizopus niveus*	64.5	0.75	0.34	0.312	10
Interspex Bacterial Esterase/Lipase BE1-Supported *P. mandocino*	44.25	0.11	>0.95	0.897	n/d
Nagase Lipase A-10 *R. japonicus*	44.25	0.72	0.73	0.504	13
Novo SP 525 Lipase, type B *C. antarctica*	122	0.77	0.18	0.191	9
Toyobo Lipoprotein lipase (LPL-701) *Pseudomonas sp.*	46.5 90*	0.95 0.957	0.62 0.486	0.395 0.337	68 74

Seikagaki Lipase *Rhizopus delemar*	122	0.69	0.63	0.478	10
Toyobo Lipoprotein Lipase (LPL-311) Type A *Pseudomonas sp.*	46.5 90*	0.97 0.975	>0.97 0.709	0.507 0.421	n/d 165
Kinzie & Payne Lipase WT *Rhizopus sp.*	138.25	0.72	0.41	0.359	9
Svedas Lipase *Rhizopus oryzae*	119	0.70	0.73	0.511	12
Sawa Lipase A-10 *Rhizopus japonicus*	47	0.68	0.82	0.547	13
Sawa LPL-701 *Pseudomonas sp.*	90*	0.967	0.467	0.326	93
Boehringer-Mannheim Chirazyme™ L2, lipase B *Candida antarctica*	47	0.82	0.05	0.060	11
Boehringer-Mannheim Chirazyme™ L4 *Pseudomonas sp.*	119	0.94	0.24	0.206	38
Boehringer-Mannheim Chirazyme™ L6 *Pseudomonas sp.*	47 90*	0.95 0.97	0.77 0.46	0.450 0.321	86 103
Interspex Lipase/Esterase ICS-16-FL1Fungal *Rhizopus oryzae*	119	0.73	0.64	0.465	12
Fluka Lipase *Aspergillus niger*	71	0.44	0.43	0.495	4
Novo Lipozyme IM-60 *Mucor miechei*	141.5	0.49	0.15	0.235	3
Sigma Lipase Type XI *Rhizopus arrhizus*	136.5	0.75	0.35	0.316	10

* Conditions: Ester (50 mg), Enzyme (50 mg), TBME/Phosphate Buffer (pH 7)(1 mL:1 mL), 300 rpm, RT.
#Conditions: Ester (19 mg), Enzyme (1 mg), Tetrahydrofuran/0.5 M MOPS buffer pH 7.0 (0.2/1.0 mL)

A similar procedure was conducted using benzyl protected racemic *trans* lactam trifluoroethyl ester I. Enzyme reactions were conducted using a two-phase system of 1.0 ml TBME with 1.0 ml of 0.1 M phosphate buffer (pH 7.0). Approximately 50 mg of enzyme and 50 mg of ester were added to the suspension and mixed with agitation (300

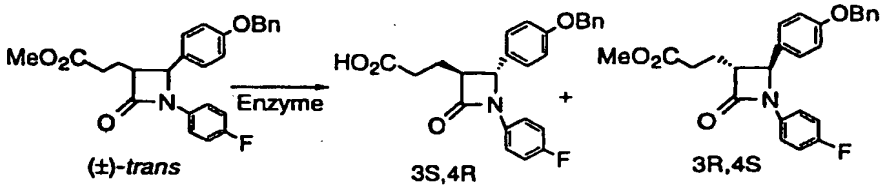
rpm) at room temperature for up to 186 hours. Material was recovered by separating the phases by centrifugation and product and unreacted starting material were analyzed by chiral HPLC. Enzymes demonstrating selective hydrolysis of racemic *trans* lactam trifluoroethyl ester I yielding acid IIb and ester Ib are summarized in Table 4.

Table 4. Enzymatic resolution of benzyl protected racemic *trans* lactam trifluoroethyl ester I yielding optically enriched ester Ib and acid IIb.

Enzyme	Time hour	(3R,4S) Ib ee_s	(3S,4R) IIb ee_p	Conversion	E
Toyobo LIP-301 *Pseudomonas sp.*	186	0.755	0.987	0.433	360
Toyobo LPL-701 *Pseudomonas sp.*	48	0.713	0.774	0.480	16
Toyobo LPL 311 (Type A) *Pseudomonas sp.*	48 24	0.994 0.991	0.628 0.788	0.613 0.557	23 44
Boehringer-Mannheim Chirazyme™ L6 *Pseudomonas sp*	48	0.680	0.796	0.461	18

Example 6

Milligram quantities of ester Ib derived from the enzymatic resolution of benzyl protected racemic *trans* lactam methyl ester were prepared as described below.



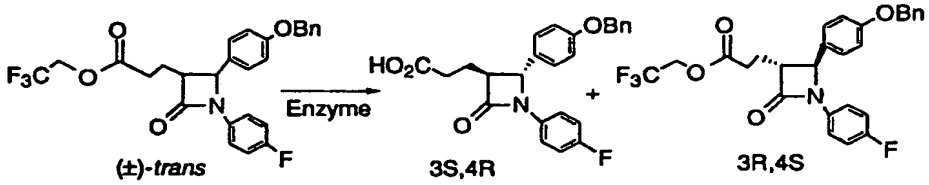
Toyobo LPL-311 (Type A) (*Pseudomonas sp.*) (202 mg) was dissolved in 0.1M phosphate buffer (pH 7) (8 mL) at room temperature. A solution of racemic methyl ester (199.5 mg, 0.46 mmol) in TBME (8 mL) was added. The two-phase mixture was shaken at 37°C at 250 rpm for 187 h. The reaction mixture was acidified with 0.5 M H_2SO_4 (1 mL), diluted with water (15 mL) and placed in two centrifuge tubes. EtOAc (20 mL) was added to each tube and the tubes shaken, then centrifuged

at 3000 rpm for 0.5 h. The organic layer was removed and the extraction/centrifugation repeated twice. The combined organic extracts were evaporated and the crude product was placed on a silica gel column (Selecto 32-63 mesh; 20 g) and eluted with 30% (300 mL) and 50% (400 mL) EtOAc/heptane, collecting fractions of ~20 mL. Fractions 4-6 were combined and evaporated to yield the (3R,4S)-methyl ester: 89 mg, 44.6%; 95.1% enantiomeric excess; $[\alpha]_D^{25} = -14.15°$ (c = 0.89, ethanol). Fractions 11-19 provided the (3S,4R)-acid: 39 mg, 20.2%; 84.5% enantiomeric excess; $[\alpha]_D^{25} = +14.87°$ (c = 0.39, ethanol).

Example 7

Milligram quantities of acid IIa derived from the enzymatic resolution of benzyl protected racemic *trans* lactam trifluoromethyl ester, followed by hydrolysis of the trifluoromethyl ester, were prepared as described below.

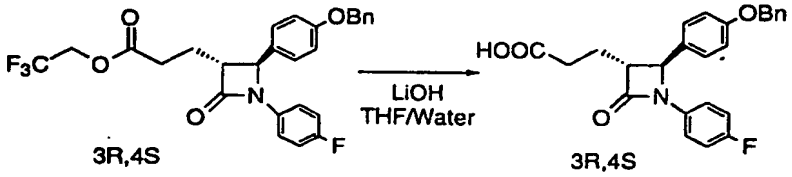
Step 1:



Toyobo LPL-311 (Type A) (*Pseudomonas sp.*) (365 mg) was dissolved in 0.1M phosphate buffer (pH 7) (16 mL) at room temperature. A solution of racemic trifluorethyl ester (428 mg, 0.85 mmol) in TBME (16 mL) was added. The two-phase mixture was shaken at 37°C at 250 rpm for 7.75 h, then stored in a refrigerator overnight. The reaction mixture was acidified with 0.5 M H_2SO_4 (1 mL), diluted with water (50 mL) and placed in four centrifuge tubes. EtOAc (15 mL) was added to each tube and the tubes shaken, then centrifuged at 3000 rpm for 0.5 h. The organic layer was removed and the extraction/centrifugation repeated twice. The combined organic extracts were evaporated and the crude product was placed on a silica gel column (Selecto 32-63 mesh; 35 g) and eluted with 30% (450 mL) and 50% (600 mL) EtOAc/heptane,

collecting fractions of ~20 mL. Fractions 5-7 were combined and evaporated to yield the (3R,4S)-trifluoroethyl ester: 191 mg, 44.6%; 99.0% enantiomeric excess; $[\alpha]_D^{25} = -9.31°$ (c = 1.88, ethanol). Fractions 18-36 provided the (3S,4R)-acid: 100 mg, 27.9%; 88.3% enantiomeric excess; $[\alpha]_D^{25} = +15.96°$ (c = 0.99, ethanol).

Step 2:

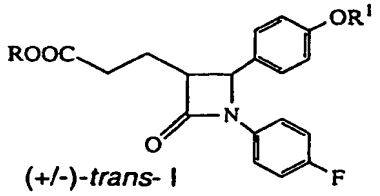


(3R,4S)-Trifluoroethyl ester (181 mg, 0.36 mmol) (99.0% ee) was dissolved in THF (4 mL) and cooled to 0°C in an ice bath. A solution of LiOH (52.5 mg, 1.25 mmol) was added and the mixture stirred at 0°C for 3.25 h, by which time HPLC indicated complete hydrolysis. The reaction mixture was acidified with 0.5 M H_2SO_4 (12 mL) and extracted with EtOAc (2X15 mL). The combined organic extracts were washed with sat'd. NaCl solution (10 mL), dried (Na_2SO_4), filtered and evaporated: 146 mg, 96.4%; 98.2% enantiomeric excess.

A sample of the crude product was purified by preparative TLC (Analtech Uniplate Silica Gel GF; 20 X 20 cm; 1000μm) eluting with 50% EtOAc/heptane: $[\alpha]_D^{25} = -16.52°$ (c = 0.66, ethanol).

WHAT IS CLAIMED IS:

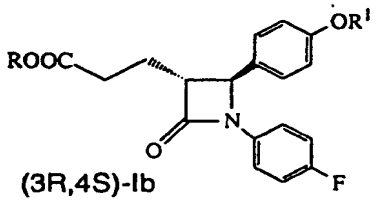
1. A process for the microbiological or enzymatic hydrolytic resolution of a racemic *trans*-2-(alkoxycarbonylethyl)-lactam of the formula I



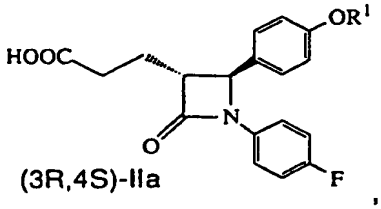
(+/-)-*trans*- I

wherein R is C_1-C_7 alkyl, 2,2,2-trifluoroethyl or methoxyethoxyethyl and R^1 is hydrogen or a protecting group selected from the group consisting of benzyl, trimethylsilyl, t-butyldimethylsilyl and acetyl, comprising:

adding a racemic lactam I to microorganisms in medium, medium and buffer, medium and solvent, or medium and a mixture of buffer and solvent, or to enzymes in buffer, solvent, or a mixture thereof, to obtain an optically enriched compound of the formula Ib or IIa



(3R,4S)-Ib



(3R,4S)-IIa

wherein R and R^1 are as defined above; and

hydrolysing a carboxylic acid ester of formula Ib to obtain a compound of formula IIa.

2. A process of claim 1 using microorganisms for the resolution of a racemic *trans* lactam I to obtain optically enriched acid IIa.

3. A process of claim 2 wherein R is methyl.

4. A process of claim 2 wherein the microorganism is of the genera selected from the group consisting of *Aspergillus, Bacillus, Candida,*

Cunninghamella, Debaryomyces, Mycobacterium, Paecilomyces, Penicillium, Rhodobacter, Streptomyces and *Trichothecium.*

5. A process of claim 4 wherein the microorganism is of the species selected from the group consisting of *Aspergillus alliaceus, niger, niveus* and *terreus*; *Bacillus sphaericus; Candida parapsilosis* and *rugosa*; *Cunninghamella homothallica; Debaryomyces hansenii; Mycobacterium fortuitum; Paecilomyces marquandii; Penicillium implicatum*; *Rhodobacter sphaeroides; Streptomyces spectabilis;* and *Trichothecium roseum.*

6. A process of claim 1 using microorganisms for the resolution of a racemic *trans* lactam I to obtain optically enriched carboxylic acid ester Ib, followed by hydrolysis to obtain optically enriched acid IIa.

7. A process of claim 6 wherein R is methyl and R^1 is benzyl.

8. A process of claim 6 wherein the microorganism is of the genera selected from the group consisting of *Comamonas, Curvularia, Mucor, Nocardia* and *Rhodococcus.*

9. A process of claim 8 wherein the microorganism is of the species selected from the group consisting of *Comamonas testosteroni; Curvularia brachyspora* and *geniculata; Mucor circinelloides* and *racemosus; Nocardia corallina;* and *Rhodococcus erythropolis, rhodochrous* and *species.*

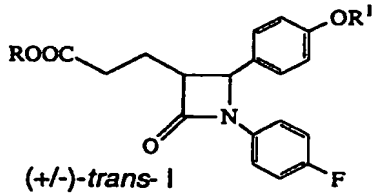
10. A process of claim 1 using enzymes for the resolution of benzyl protected racemic *trans* lactam I to obtain optically enriched carboxylic acid ester Ib, followed by hydrolysis to obtain optically enriched acid IIa.

11. A process of claim 10 wherein R is methyl or trifluoroethyl and R^1 is benzyl.

12. A process of claim 10 wherein the enzyme is selected from the group consisting of a hydrolase from *Rhizopus delemar, Rhizopus javanicus, Mucor javanicus, Rhizopus niveus, Pseudomonas mandocino, Rhizopus japonicus, Candida antarctica ,Pseudomonas sp., Rhizopus sp., Rhizopus oryzae, Aspergilius niger, Mucor miehei,* and *Rhizopus arrhizus.*

13. A process of claim 12 wherein the enzyme is selected from the group consisting of a hydrolase from a *Pseudomonas species.*

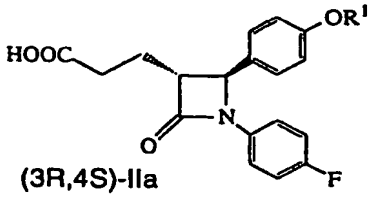
14. A process for the hydrolytic resolution of a racemic *trans*-2-(alkoxycarbonylethyl)-lactam of the formula I



(+/-)-*trans*- I

wherein R is C_1-C_7 alkyl, 2,2,2-trifluoroethyl or methoxyethoxyethyl and R^1 is hydrogen or a protecting group selected from the group consisting of benzyl, trimethylsilyl, t-butyldimethylsilyl and acetyl, comprising:

adding a racemic lactam I to a microorganism of the genera selected from the group consisting of *Aspergillus, Bacillus, Candida, Cunninghamella, Debaryomyces, Mycobacterium, Paecilomyces, Penicillium, Rhodobacter, Streptomyces* and *Trichothecium* in medium, medium and solvent, medium and buffer, or medium and a mixture of buffer and solvent, to obtain an optically enriched compound of the formula

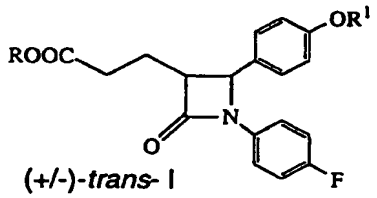


(3R,4S)-IIa

wherein R^1 is as defined above.

15. A process of claim 14 wherein the microorganism is *Aspergillus alliaceus* or *terreus*, or *Candida parapsilosis*.

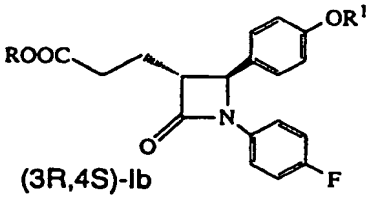
16. A process for the hydrolytic resolution of a racemic *trans*-2-(alkoxycarbonylethyl)-lactam of the formula I



(+/-)-*trans*- I

wherein R is C_1-C_7 alkyl, 2,2,2-trifluoroethyl or methoxyethoxyethyl and R^1 is hydrogen or a protecting group selected from the group consisting of benzyl, trimethylsilyl, t-butyldimethylsilyl and acetyl, comprising:

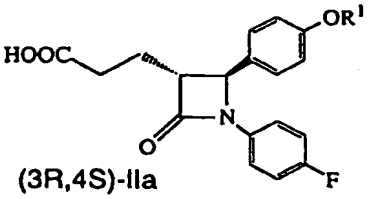
adding a racemic lactam I to a microorganism of the genera selected from the group consisting of *Comamonas, Curvularia, Mucor, Nocardia* and *Rhodococcus* in medium, medium and solvent, medium and buffer, or medium and a mixture of buffer and solvent, to obtain an optically enriched compound of the formula Ib



(3R,4S)-Ib

wherein R and R^1 are as defined above; and

hydrolysing the carboxylic acid ester of formula Ib to obtain a compound of formula IIa

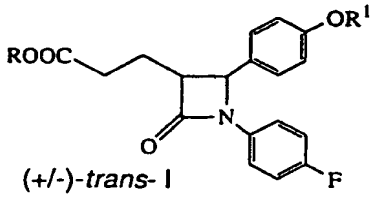


(3R,4S)-IIa

wherein R^1 is as defined above.

17. A process of claim 16 wherein the micoorganism is *Rhodococcus rhodochrous* or *species*

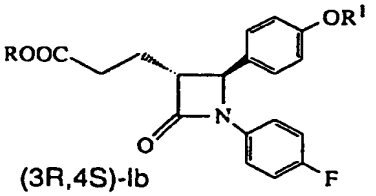
18. A process for the hydrolytic resolution of a racemic *trans*-2-(alkoxycarbonylethyl)-lactam of the formula I



(+/-)-*trans*- I

wherein R is C_1-C_7 alkyl, 2,2,2-trifluoroethyl or methoxyethoxyethyl and R^1 is hydrogen or a protecting group selected from the group consisting of benzyl, trimethylsilyl, t-butyldimethylsilyl and acetyl, comprising:

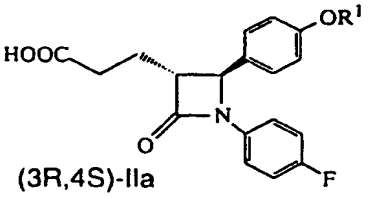
adding a racemic lactam I to an enzyme selected from the group consisting of a hydrolase of *Pseudomonas species* in buffer, solvent, or a mixture thereof, to obtain an optically enriched compound of the formula Ib



(3R,4S)-Ib

wherein R and R^1 are as defined above; and

hydrolysing the carboxylic acid ester of formula Ib to obtain a compound of formula IIa



(3R,4S)-IIa

wherein R^1 is as defined above.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/21436

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C12P 41/00, C12P 17/10
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 9941405 A1 (G.D. SEARLE & CO.), 19 August 1999 (19.08.99), page 2 - page 3 --	1-18
X	Tetrahedron Letters, Volume 38, No 4, 1997, AMIT BASAK ET AL, "Differential Effects of Stereochemistry and C-4 Substituents on the Enantioselectivity of PLE and PPL Catalysed Hydrolysis of 3,4-Disubstituted -lactams", page 643 - page 646, see p. 644, 5-8 --	1-18
A	US 5811292 A (R.N. PATEL ET AL), 22 Sept 1998 (22.09.98) -- --------	1-18

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 January 2000	10.02.00
Name and mailing address of the International Searching Authority European Patent Office P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel: +31-70-340-2040. Tx 31 651 epo nl Fax: +31-70-340-3016	Authorized officer Anna Sjölund / MR Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US 99/21436

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9941405 A1	19/08/99	NONE	
US 5811292 A	22/09/98	US 5567614 A	22/10/96
		CA 2087359 A	16/07/93
		EP 0552041 A	21/07/93
		JP 5308996 A	22/11/93

L10 ANSWER 1 OF 2 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN
ACCESSION NUMBER: ABG22702 Protein DGENE
TITLE: New isolated polynucleotide and encoded polypeptides, useful
in diagnostics, forensics, gene mapping, identification of
mutations responsible for genetic disorders or other traits
and to assess biodiversity -
INVENTOR: Drmanac R T; Liu C; Tang Y T
PATENT ASSIGNEE: (HYSE-N)HYSEQ INC.
PATENT INFO: **WO 2001075067 A2 20011011 103p**
APPLICATION INFO: WO 2001-US8631 20010330
PRIORITY INFO: US 2000-540217 20000331
US 2000-649167 20000823
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 2001-639362 [73]
CROSS REFERENCES: N-PSDB: AAS86889
DESCRIPTION: Novel human diagnostic protein #22693.
SEQ

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L10 ANSWER 2 OF 2 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN
ACCESSION NUMBER: AAS86889 cDNA DGENE
TITLE: New isolated polynucleotide and encoded polypeptides, useful
in diagnostics, forensics, gene mapping, identification of
mutations responsible for genetic disorders or other traits
and to assess biodiversity -
INVENTOR: Drmanac R T; Liu C; Tang Y T
PATENT ASSIGNEE: (HYSE-N)HYSEQ INC.
PATENT INFO: **WO 2001075067 A2 20011011 103p**
APPLICATION INFO: WO 2001-US8631 20010330
PRIORITY INFO: US 2000-540217 20000331
US 2000-649167 20000823
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 2001-639362 [73]
CROSS REFERENCES: P-PSDB: ABG22702
DESCRIPTION: DNA encoding novel human diagnostic protein #22693.
SEQ

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